

Molecular Cloning

A LABORATORY MANUAL

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Back cover: *E. coli* DH1 with fimbriae was negatively stained with phosphotungstic acid and the electron micrograph was digitized and assigned false color by computer. *Jeffrey A. Engler, Thomas R. Broker, and James I. Garrels*

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HYBRIDIZATION OF SOUTHERN FILTERS

1. Float the baked filter on the surface of 6× SSC until it wets from beneath. Immerse the filter in the 6× SSC for 2 minutes.
2. Slip the wet filter into a heat-sealable plastic bag (e.g., Sears' Seal-n-Save).
3. Add 0.2 ml of prehybridization fluid warmed to 68°C for each square centimeter of nitrocellulose filter.

Prehybridization fluid

6× SSC
 0.5% SDS
 5× Denhardt's solution (see page 448)
 100 µg/ml denatured, salmon sperm DNA (see page 327)

4. Squeeze as much air as possible from the bag. Seal the open end of the bag with the heat sealer. Incubate the bag for 2–4 hours submerged in a water bath at 68°C.

Often, small bubbles of air form on the surface of the filter as the temperature of the prehybridization solution rises to 68°C. It is important that these bubbles be removed by occasionally agitating the fluid in the bag; otherwise the components of the prehybridization fluid will not be able to coat the filter evenly.

5. Remove the bag from the water bath. Open the bag by cutting off one corner with scissors. Squeeze out as much prehybridization solution as possible.
6. Using a pasteur pipette, add the hybridization solution to the bag. Use just enough solution to keep the filter wet (50 µl/cm² of filter).

Hybridization solution

6× SSC
 0.01 M EDTA
³²P-labeled denatured probe DNA
 5× Denhardt's solution
 0.5% SDS
 100 µg/ml denatured, salmon sperm DNA

Typical hybridization conditions for Southern filters are given in Table 11.1.

7. Squeeze as much air as possible from the bag. Seal the cut edge with the heat sealer so that as few air bubbles as possible are trapped in the bag.

TABLE 11.1 HYBRIDIZATION CONDITIONS FOR SOUTHERN FILTERS

DNA on filter	Sp. act. of probe DNA (cpm/ μ g)	Amount of probe added	Time of hybridization (hr)
Fragments of cloned DNA (~100 ng/fragment)	10^7	10^5 – 10^6 cpm (0.01–0.1 μ g)	3–4
Total eukaryotic DNA (10 μ g)	10^8	1×10^7 cpm – 5×10^7 (0.1–0.5 μ g)	12–16

8. Incubate the bag submerged in a water bath at 68°C for the required hybridization period.

9. Remove the bag from the water bath and quickly cut along the length of three sides. Using gloves, remove the filter and immediately submerge it in a tray containing a solution of 2 \times SSC and 0.5% SDS at room temperature.

Note. Do not allow the filter to dry out at any stage during the washing procedure.

10. After 5 minutes, transfer the filter to a fresh tray containing a solution of 2 \times SSC and 0.1% SDS and incubate for 15 minutes at room temperature with occasional gentle agitation.

11. Transfer the filter to a flat-bottomed plastic box containing a solution of 0.1 \times SSC and 0.5% SDS. Incubate at 68°C for 2 hours with gentle agitation. Change the buffer and continue incubating for a further 30 minutes.

Note. If the homology between the probe and the DNA bound to the filter is inexact, the washing should be carried out under less stringent conditions. In general, washing should be carried out at $T_m = -12^\circ\text{C}$.

The following relationships are useful:

a. $T_m = 69.3 + 0.41 \cdot (G + C)\%$ (Marmur and Doty 1962)

b. The T_m of a duplex DNA decreases by 1°C with every increase of 1% in the number of mismatched base pairs (Bonner et al. 1973).

c. $(T_m)_{\mu_2} - (T_m)_{\mu_1} = 18.5 \log_{10} \frac{\mu_2}{\mu_1}$

where μ_1 and μ_2 are the ionic strengths of two solutions (Dove and Davidson 1962).

12. Dry the filter at room temperature on a sheet of Whatman 3MM paper.
13. Wrap the filter in Saran Wrap and apply to X-ray film to obtain an autoradiographic image (see page 470).

Notes

Hybridization may also be carried out in:

- a. flat-bottomed plastic boxes.
- b. buffers containing formamide. Each increase of 1% in the formamide concentration lowers the T_m of a DNA duplex by 0.7°C (McConaughy et al. 1969; Casey and Davidson 1977).

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